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Degradation of eight relevant micropollutants in different water matrices by neutral photo-Fenton process under UV_{254} and simulated solar light irradiation – A comparative study

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ABSTRACT

This is a comparative study of photolytic degradation under exposure to UV_{254} nm and solar-simulator irradiation of a mixture of eight equally concentrated micropollutants in the presence of H_2O_2 and Fe(II) in ultra-pure water, Lake Geneva water, and effluent from a wastewater treatment plant (WWTP).

The electron spin resonance experiments point to a low singlet oxygen formation efficiency by the micropollutant mixture. This finding corroborates the micropollutants' chemical stability under UVC irradiation (in decreasing order: gabapentin, metformin, metoprolol, atenolol, clarithromycin, primidone, methylbenzotriazole, and benzotriazole). The oxidation rate increased in the presence of low-concentration H_2O_2 and Fe(II), except for metformin and gabapentin. Gabapentin and metformin were the most persistent compounds, with less than 24% being removed after 60 min of UV_{254}/H_2O_2/Fe(II) treatment. The low removal rates were observed in WWTP effluent and lake water, and using sunlight simulation.

Guanylurea, phenol, oxalic acid, tartronic acid, glycolic acid, oxamic acid, and maleic acid, could also be detected as fragmental oxidation products. Furthermore, up to 300 μg/L of nitrate and ammonia were identified as final degradation products. Ecotoxicological tests showed that the degradation products are more toxic for algae *Chlamydomonas reinhardtii* than the parent compounds themselves.

1. Introduction

The large number of registered chemicals (more than 100,000 substances, under REACH regulation [1]), along with their diversity, adds to water contamination's complexity. Numerous studies have reported a great variety of pharmaceuticals and personal care products (PPCPs) in concentrations of ng/L to μg/L in sewage treatment effluents, rivers, surface and ground waters, and drinking water [2–6]. Even at low concentrations, these pollutants may still have chronic effects if continuously released into the environment [7]. Because of the large volumes of micropollutants released into the environment, as well as their bio-persistence and bio-accumulation, their removal by conventional biological, physical, and chemical methods is difficult and costly. Therefore, these compounds should be removed from water supplies before being discharged into general aquatic environments. There are four approaches to removing micropollutants: (i) optimize existing technology at wastewater treatment plants (WWTPs), (ii) upgrade WWTPs with new technologies, (iii) control the water pollution source, and (iv) separate the source.

The main focus is usually on end-of-pipe measurements. However, the removal of all compounds is often not satisfactory. Pharmaceuticals do not occur in the environment as single

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contaminants, but rather as a complex mixture of various PPCPs and contaminants, such as pesticides or industrial chemicals [2]. The overall environmental toxicity of a complex mixture of pollutants may be much stronger than individual chemical agents' toxic effect [8]. The stability and toxicity of transformation products formed during treatment and environmental transport are also important when determining the risks associated with the presence of active pharmaceuticals [9]. Donner et al. [9] showed that the carbamazepine transformation products were considerably more toxic than the parent compound itself and might be generated during wastewater effluent UV treatment and/or pharmaceuticals' photo-induced degradation in natural waters. Although the individual investigation of widely used pharmaceuticals is relatively well-documented, their toxic properties in a mixture, as occurs in the environment, have not been thoroughly investigated.

This study highlights the need to consider the behavior of a mixture of compounds in different water matrices and different experimental conditions. Eight widely used micropollutants that are frequently found in water effluents were selected for study: Atenolol, benzotriazole, clarithromycin, gabapentin, methylbenzotriazole, metformin, metoprolol and primidone. The choice of these compounds was based on consumption consideration, their trace-level toxicity (ng/L) for aquatic flora [5,8] and fauna, persistence in the environment, and stability after municipal WWTP processing [10–13].

Given the selected compounds' impact and the insufficiency of current decontamination approaches, enhanced technologies are needed. Our goal was to compare relevant micropollutants' behavior at the same molar concentration by UV_{254}, UV_{254}/H_2O_2, $UV_{254}/H_2O_2/Fe(II)$, and solar-simulated light//H_2O_2/Fe(II) in different water matrices; and to investigate a treatment method that can be integrated into water and wastewater facilities to prevent releasing selected compounds into natural waters. The effect of hydrogen peroxide, iron addition, light and contact time, and water constituents on the degradation of compounds and toxicity during the treatment have been investigated.

2. Materials and methods

2.1. Reagents

The high-purity micropollutants, deuterated standards, and 2,2,6,6-tetramethyl-4-piperidinol were purchased from Sigma–Aldrich (Buchs, Switzerland), Dr. Ehrenstorfer GmbH (Augsburg, Germany), and Toronto Research Chemicals (North York, Canada). Table S1 (Supporting Information) shows the compounds' main characteristics. Solvents were of HPLC GOLD quality (Carlo Erba Reagent, Italy). Water for UPLC–MS/MS was ultrapure (MilliQ, Millipore). Hydrogen peroxide (Sigma–Aldrich), ferrous sulfate heptahydrate (Fluka Chemika), and all chemicals used for solutions (such as buffer, eluents) were reagent grade and used without further purification.

Natural organic matter (NOM) was taken from Lake Geneva (Switzerland) and sampled on March 10, 2013. The effluent from the Lausanne WWTP was taken on March 18, 2013. Table S2 shows the measured basic characteristic data of the Lake Geneva water and WWTP effluent.

The 24-h composite sample (60 ml taken every 15 min, time proportional) was collected with a refrigerated, automatic sampler (ISCO 6712 FR, Teledyne, US) at the effluent of the moving bed bioreactor (MBBR) with nitrification (BIO). The composite sample was stored at 4 °C.

2.2. Sample preparation

Solutions containing a micropollutant concentration of 2.00 μmol L^{-1} were prepared by adding an appropriate volume of methanol stock solution to ultrapure water for the final concentration. Canonica et al. [14,15] found that the methanol content of aqueous solutions (up to 2%) had no significant influence on photo-oxidation experiments. In our experiments, the methanol content in the final solution was 0.5%. For experiments with a model solution containing NOM, a mixture of lake water, WWTP effluent, and the stock solutions was used to get the same final micropollutant concentration. For UPLC/MS–MS analyses, the filtered samples were diluted in eluent A (Table S3, Supporting Information), containing a mixture of deuterated standards.

Solid-phase extraction (SPE) using Oasis HLB extraction cartridges, (V = 6 cm^3, sorbent mass m = 200 mg, Waters, US), was performed prior to degradation product UPLC–MS/MS analyses (Supporting Information).

Residual hydrogen peroxide was removed by adding manganese dioxide. Samples of the reaction medium were withdrawn at regular intervals. The reaction was then blocked by raising the pH to 9–10, adding MnO_2, and allowing the samples to sit overnight. No concentration changes were observed by performing a blank experiment. The manganese dioxide remained unchanged at the end of the experiment.

2.3. Analyses of micropollutants

Sample analysis was performed by UPLC–MS/MS (Acquity TQD, Waters) and is presented in detail in Supporting Information (Tables S3 and S4).

Total organic carbon was determined using a Shimadzu model TOC-V_{CPH} analyzer, based on combustion catalytic oxidation. The samples were previously acidified with HCl, degassed with O_2, and the purgeable organic and inorganic carbon were eliminated. COD determination was conducted using commercially available test kits (Machery and Nagel, Düren, Germany) for solutions with no significant chloride content. The pH and conductivity were respectively measured by a Metrohm pH-meter 780 and Hach Conductometer Model 44600.

UV–vis absorption spectra of Lake Geneva water and WWTP effluent were recorded using a Hitachi U-2001 spectrophotometer, in a 1 cm pathlength quartz cuvette.

Anion analysis was conducted by a Dionex model ICS-3000 ion chromatograph (Dionex, Sunnyvale, CA, US) equipped with an IonPac™ AS11 HC column (250 mm × 4 mm ID) and operating in suppressed conductivity detection mode. Samples, injected at a volume of 25 μl by an automatic sampler, were eluted by 30 mM NaOH gradient at a flow rate of 1.0 ml/min.

2.4. Irradiation experiments

The photodegradation experiments were carried out in a stirred, batch cylindrical water-jacketed glass photoreactor (irradiated solution volume = 400 ml, optical path length = 1.7 cm) at 295 K. The incident photonic flux ($P_0 = 4.39 \times 10^{-6}$ einstein s^{-1}) was measured by hydrogen peroxide actinometry. The detailed description of the UVC reactor and the solar simulator were presented in Supporting Information and elsewhere [10].

2.5. Electron spin resonance experiments

Before electron spin resonance (ESR) detection of the photosensitized $^1\Delta_g$, the 2 mL-sample volumes of aqueous suspensions containing the eight pharmaceutical compounds and 20 mM of 2,2,6,6-tetramethyl-4-piperidinol (TMP-OH) were transferred into

a small (5 ml) Pyrex beaker and exposed to either UVA (λ = 365 nm, 2.5 mW/cm^2, from a UV spot light source) (Lightingcure^{TM} LC-8, Hamamatsu Photonics, France) or UVC (λ = 254 nm, 4.75 mW/cm^2, from a low-pressure mercury lamp) (Pen-Ray, Upland, CA, US) light. The illumination was performed at a fixed temperature of 36 °C in a thermostated, custom-built photoreactor. During illumination, the suspensions were equilibrated with oxygen at the atmospheric pressure and magnetically stirred.

For acquiring ESR traces, small aliquots of approximately 20 μl of illuminated suspensions were transferred into 0.7 mm ID and 0.87 mm OD glass capillary tubes (VitroCom, NJ, US), with a sample height of approximately 40 mm, and sealed on both ends with Chaseal (tube-sealing compound, Chase Scientific Glass, Rockwood, TN, US) and positioned in the active zone of the ESR spectrometer TE_{102} cavity (Model ESP300E, Bruker BioSpin, Germany). The typical instrumental settings were: Microwave frequency 9.78 GHz, microwave power 2.0 mW, sweep width 120 G, modulation frequency 100 kHz, modulation amplitude 0.5 G, receiver gain 2×10^4, time constant 10.24 ms, conversion time 40.96 ms, number of spectral points 2048, and total scan time 83.9 s.

2.6. Toxicity testing

To examine the phytotoxicity of micropollutant mixture solutions before and after irradiation treatments, the unicellular green alga *Chlamydomonas reinhardtii* P.A. Dangeard (strain CPCC 11) was used as the model organism. The alga was obtained from the Canadian Phycological Culture Centre (CPCC, Department of Biology, University of Waterloo, Waterloo, ON, Canada) and cultured axenically in a 4-fold diluted TAP [16] at pH 7 under constant illumination at 70 μE m^{-2} s^{-1} (cool white fluorescent tubes), with rotary agitation at 100 rpm and at a temperature of 20 °C.

At mid-exponential growth phase, algal cells were harvested by centrifugation (3000 × g, 10 min), rinsed with OECD medium [17], which was previously enriched with 10 mM of 3-(N-morpholino)propanesulfonic acid (MOPS) at pH 7, and inoculated into experimental media to obtain a final cell density of 1×10^5 cells/mL. Exposure experiments were performed in 96 well plates to which 190 μL of experimental media and 10 μL of concentrated algal solutions were added. Experimental media to be tested, diluted between 50- and 2-fold with OECD medium, were composed of (i) a mixture of eight micropollutants (atenolol, benzotriazole, clarithromycin, gabapentin, methylbenzotriazole, metformin, metoprolol and primidone; 2 μM final concentration), and (ii) a mixture of four micropollutants (gabapentin, metformin, benzotriazole and diclofenac; 2 μM final concentration), which were both diluted in milliQ water or waste water and which were treated with UV/H_2O_2 (300 μM) – no Fe(II) added and UV/H_2O_2 (300 μM) – 30 μM Fe(II) added. When no dilution was applied, concentrated stock solutions used to prepare OECD medium were directly added to the mixture solutions to be tested. Control media (without micropollutants) were also prepared by the direct addition of stock solutions to milliQ water or wastewater.

After 72 h of exposure, algal cell density was measured by flow cytometry (BD Accuri^{TM}C6, BD Biosciences, San Jose, US) and the percent yield of inhibition (%I) was calculated following the Eq. (1):

$$\%I = \frac{Y_c - Y_T}{Y_C} \times 100 \quad (1)$$

where Y_c is the mean value for the control group yield and Y_T is the mean value for the treated group yield.

The test solution percentages affecting 50 percent of algal yield (EC50) as well as the 95 percent confidence intervals (CI_{95}) were calculated using the excel macro REGTOX (http://www.normalesup.org/ vindimian/fr_index.html) set with the Hill approach and a bootstrap nonparametric simulation.

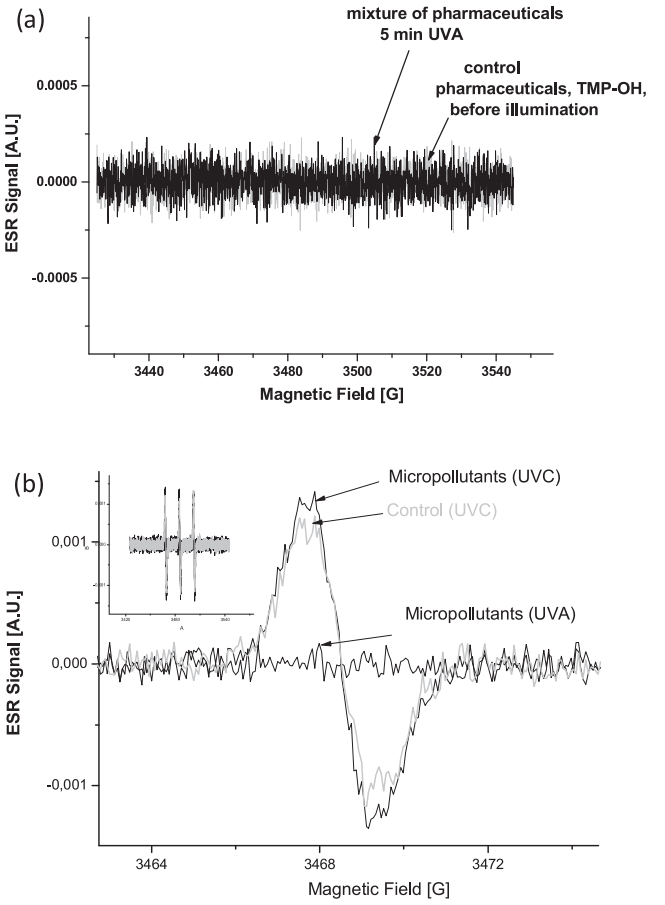

Fig. 1. The ESR signals of TEMPOL acquired after five minutes of UVA illumination (λ = 365 nm) (a) and after 15 min of UVC illumination (λ = 254 nm) (b) for the mixture of eight micropollutants and the control solution containing only 20 mM TMP-OH. Inset: the corresponding ESR spectra acquired in this experiment.

3. Results and discussion

3.1. ESR detection of singlet oxygen

Singlet molecular oxygen (denoted as $^1\Delta_g$), the lowest, electronic-excited state of molecular oxygen, is an important agent in a number of chemical processes. It is also one of the main activated species responsible for light-induced damaging effects in biological systems. This is particularly true in so-called photodynamic oxidation processes, where $^1\Delta_g$ is generated by a very efficient energy transfer from the light-excited organic molecule (photosensitizer) in its long-lived excited triplet state to the dissolved, ground triplet molecular oxygen, $^3\sum_g$ [18].

ESR was employed to follow the formation and quenching of the photosensitized $^1\Delta_g$ in aqueous solutions containing the selected compounds. In this approach, first introduced by Lion et al. [19], the diamagnetic scavenger, 2,2,6,6-tetramethyl-4-piperidinol (TMP-OH), reacts with $^1\Delta_g$, yielding a stable paramagnetic product, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL). This product can easily be detected by ESR. Although other ROS can also react with TMP-OH, leading to formation of TEMPOL [20], the reactive scavenging of $^1\Delta_g$ by TMP-OH is considered a highly $^1\Delta_g$-specific process [21]. As Fig. 1a shows, no marked ESR signal could be observed for the micropollutant mixture exposed to UVA illumination for five minutes.

In contrast, after 15-min exposure to UVC light, the solution containing the mixture of eight micropollutants, as well as the control

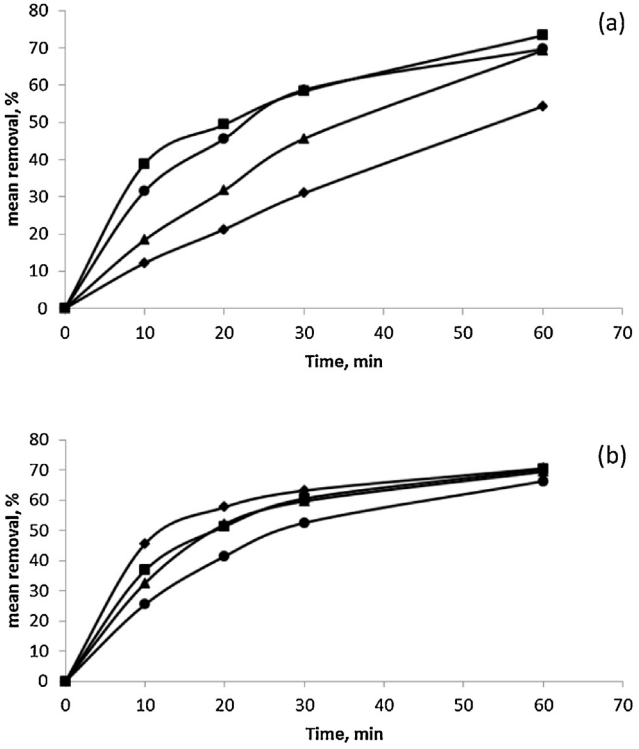

Fig. 2. Effect of hydrogen peroxide concentration (a) (♦ – UV; ■ – UV/300 μM H_2O_2; ▲ – UV/450 μM H_2O_2; • – UV/600 μM H_2O_2) and of adding Fe(II) (b) upon the photodegradation of compounds in the ultrapure water (♦ – UV/300 μM H_2O_2/30 μM Fe(II); ■ – UV/300 μM H_2O_2/60 μM Fe(II); ▲ – UV/600 μM H_2O_2/30 μM Fe(II); • – UV/600 μM H_2O_2/60 μM Fe(II)). Initial conditions: 2 μM of each pollutant in milliQ water, pH 6.50 at 295 K.

solution (containing only TMP-OH), showed a characteristic ESR signal (Fig. 1b inset).

This ESR signal, consisting of three equidistant and equiintense hyperfine lines (Fig. 1b, inset), with spectral parameters ΔH_{pp} = 1.58 G, g = 2.00570, and a_N = 17.13 G, indicates TEMPOL. Under UVC irradiation, a low production of singlet oxygen species was observed for both the mixture of eight compounds and the control solution.

As can also be seen in Fig. 1b, the ESR signal TEMPOL amplitude acquired for the mixture of eight micropollutants is slightly larger (by approximately 8%) than for the control solution. This might suggest that, under exposure to UVC light, the micropollutant mixture slightly contributed to the overall singlet oxygen formation.

Nevertheless, the ESR experiments point to rather low efficiency for singlet oxygen formation by the micropollutant mixture. This finding corroborates the observed chemical stability of the studied compounds under UVC irradiation, in the absence of hydrogen peroxide and Fe(II). Therefore, compound degradation requires additional ROS generation mechanisms, such as forming hydroxyl radicals in the presence of hydrogen peroxide and Fe(II).

3.2. Influence of hydrogen peroxide concentration on the oxidation of micropollutants

The goal of this study was to simulate degradation processes that might be used during water treatment. We irradiated compounds and different hydrogen peroxide doses with a low-pressure mercury lamp. Figs. 2a and 3 show the results.

Tables 1 and 2 show the TOC removal, pH evolution, and the first-order rate constants. Micropollutant stability during UV treatment was, in decreasing order: Gabapentin, metformin, metoprolol, atenolol, clarithromycin, primidone, methylbenzotriazole,

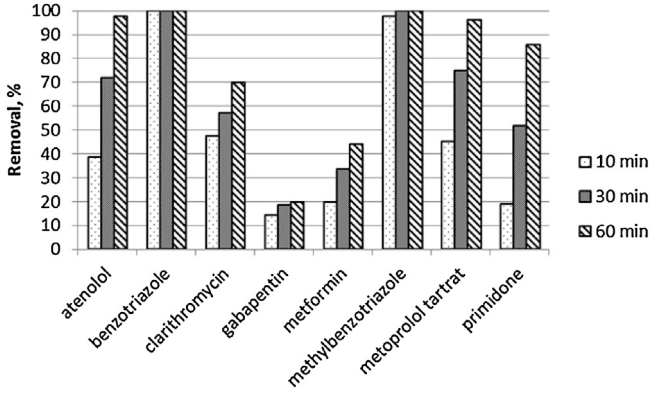

Fig. 3. Degradation of micropollutants during the UV/300 μM H_2O_2 treatment. Initial conditions: 2 μM of each pollutant in milliQ water, pH 6.50 at 295 K.

Table 1
pH evolution and TOC removal (%) of compounds upon photodegradation at different hydrogen peroxide and iron concentrations. Initial conditions: 2 μM of each pollutant in milliQ water, pH 6.50 at 295 K, COD = 3180 mg O_2/L, TOC = 1540 mg C/L.

Process	TOC removal (%)[a]	pH[a]
UV	32.47	4.30
UV/H_2O_2 (300 μM)	39.33	3.90
UV/H_2O_2 (450 μM)	45.64	4.01
UV/H_2O_2 (600 μM)	48.81	3.88
UV/H_2O_2 (300 μM)/Fe(II) (30 μM)	45.85	3.83
UV/H_2O_2 (300 μM)/Fe(II) (60 μM)	44.20	3.81
UV/H_2O_2 (600 μM)/Fe(II) (30 μM)	43.83	3.80
UV/H_2O_2 (600 μM)/Fe(II) (60 μM)	43.38	3.53

[a] After 60 min of reaction time.

and benzotriazole. Gabapentin and metformin were the most persistent compounds.

It is noteworthy that a small conversion of pollutants was observed when exposed to UV light in the absence of H_2O_2. In the presence of H_2O_2 and UV light, the conversion substantially increased due to the hydroxyl radicals generated during the photochemical reactions. After 30 min of irradiation in the absence of H_2O_2, the mean micropollutant removal rate in MilliQ water was only 30%. At the same reaction time, by adding 300 μM H_2O_2 and 600 μM H_2O_2, more than 45.55% and 58.68% of compounds were removed, respectively. After 60 min of irradiation, no essential differences in compound removal rates (approximately 70%) were seen between 300 and 600 μM H_2O_2. The first-order reaction rate benzotriazole and primidone constants were comparable with that reported by Benitez et al. [22] (k_{UV/H_2O_2} = 0.442 min^{-1}) and Real et al. [23] (k_{UV} = 0.011 min^{-1} and k_{UV/H_2O_2} = 0.063 to 0.126 min^{-1}). Even in the most beneficial case, the total

Table 2
Apparent first-order rate constants (min^{-1}) for the photodegradation of selected compounds in MilliQ water at different hydrogen peroxide concentrations.

Compounds	UV	UV/H_2O_2 (300 μM)	UV/H_2O_2 (450 μM)	UV/H_2O_2 (600 μM)
Gabapentin	0.0025	0.0049	0.0094	0.0052
Metformin	0.0037	0.0152	0.0092	0.0029
Metoprolol tartrat	0.0081	0.0515	0.0283	0.0777
Atenolol	0.0105	0.0600	0.0221	0.0661
Clarithromycin	0.0116	0.0317	0.0146	0.0149
Primidone	0.0149	0.0301	0.0135	0.0288
Methylbenzotriazole	0.0856	0.3780	0.4180	0.4120
Benzotriazole	0.3863	0.6908	0.4510	0.4962

The apparent first-order rate constants (k_{ap}) of the degradation after 60 min of reaction time were calculated from linear regression $\ln(C_0/C)$ versus time plots, with all regression coefficients greater than 0.9.

TOC removal rate was quite low (between 32% and 49%), compared to the total degradation that micropollutant content achieved (Table 1). As proposed by Kavitha and Palanivelu [24] and Mico et al. [25], the formation of hardly oxidizable by-products, such as short-chain carboxylic acids refractory to radical oxidation, deters TOC removal. Due to the production of organic and inorganic acid anions, the pH decreased from 6.50 to 3.53–4.30 during irradiation (Table 1). Micropollutant stability during UV/H_2O_2 (300 μM) treatment was, in decreasing order: Gabapentin, metformin, primidone, clarithromycin, methylbenzotriazole, metoprolol, atenolol, and benzotriazole. Gabapentin and metformin were still the most stable compounds.

The results also show the synergetic effects of H_2O_2 and UV light on the degradation and the degree of degradation dependence on the initial hydrogen peroxide concentration during the first few minutes of irradiation. It is expected that increasing the H_2O_2 concentration beyond a value higher than 300 μM will accelerate consumption of the very reactive •OH radicals to produce less reactive HO_2• radicals [26,27]. Thus, excess H_2O_2 becomes a scavenger for hydroxyl radicals. An H_2O_2 dose higher than 300 μM corresponds to unprofitable hydrogen peroxide consumption. The selection of an optimum hydrogen peroxide concentration for the substrate degradation is important from a practical standpoint due to the cost of H_2O_2. For example, the first-order rate constants from 0.005 to 0.69 min^{-1} in the presence of 300 μM H_2O_2 are more than six times greater for metoprolol and more than four times greater for metformin and methylbenzotriazole than without adding H_2O_2 (Table 2). This means that the photoreactor volume for the same water flow rate and same experimental conditions (lamp intensity and power, H_2O_2 concentration, etc.) can be more than six or four times smaller.

3.3. Influence of adding Fe(II) on selected compounds oxidation

In the next step, we investigated the influence of adding Fe(II) in the presence of H_2O_2 on selected compound oxidation at neutral pH in dark, under UV, and simulated solar irradiation. Few reports have focused on removing micropollutants at low concentrations using photo-Fenton at near-neutral pH as post-treatment for decontaminating municipal wastewater effluents treated by biological processes [10,28,29] and in waters containing dissolved organic matter (DOM) [30,31].

Several studies reported an optimum H_2O_2 dosage in Fenton reaction. The optimal ratio of chemicals in the Fenton process recommended in the literature are the ratios H_2O_2/catalyst from 10:1 to 40:1 [32]. In our experiments, the ferrous concentrations were 30 and 60 μM, with a ratio of 10:1 to 20:1. Tables 1, 3 and Fig. 2b show the results.

As expected, most of the H_2O_2 was consumed in the first stage of the fast reaction (Fig. 2b). This agreed with the Fenton and photo-Fenton reactions' dominating the first minutes of the process, and a larger reactant concentration directly increased the reaction rate [33]. By adding 30 μM Fe(II) in the presence of 300 μM H_2O_2, the mean micropollutant removal rates in ultrapure water were more than 57% after 20 min of UV_{254} irradiation, and more than 70% after 60 min of irradiation. In the absence of Fe(II), the mean removal rates were more than 49% after 20 min of reaction, and more than 73% after 60 min. Some iron complexes with organic ligands (certain by-products, such as identified oxalic acid) were probably formed during the reaction time. These complexes exhibit higher absorbance and quantum yields than simple, aquated Fe-species. Therefore, they act as a light-absorbing species in the system. The contribution of hydrogen peroxide in photo-Fenton applications was limited by the weak light absorption of H_2O_2 and the strong inner-filter effect due to light absorption by iron and organic compounds [27].

Atenolol, methylbenzotriazole, benzotriazole, and metropolol had the best degradation profiles. The presence of 300 μM of H_2O_2 and 30 μM of Fe(II) provided the highest mean removal and TOC micropollutant removal. All of them had an 80% removal rate in the first 30 min. Under the same conditions, less than 15% of gabapentin and metformin were removed. Micropollutant stability during UV/H_2O_2 (300 μM)/Fe(II)(30 μM) treatment was, in decreasing order: Metformin, gabapentin, clarithromycin, primidone, atenolol, metoprolol, methylbenzotriazole, and benzotriazole. The metformin and methylbenzotriazole degradation was lower in the presence of Fe(II). Again, the most stable compounds were gabapentin and metformin.

Pollutant degradation was also investigated using simulated solar light (Fig. S1, Supporting Information). The results show that the removal rate was significantly slower under simulated solar irradiation. This could be probably explained by the irradiation depth, the low amount of •OH radicals available to oxidize organic compounds, and the strong inner-filter effect due to light absorption by iron.

To get a more detailed picture of micropollutants' stability, the two most stable pharmaceuticals (metformin and gabapentin), and two compounds (diclofenac, an anti-inflammatory and analgesic drug, and benzotriazole) named in new legislation regarding measures to eliminate 80% of organic trace substances in WWTP (FOEN [34]), were selected for simultaneous irradiation experiments. Although diclofenac and benzotriazole were completely removed in the first 10 min of reaction during UV/H_2O_2 (300 μM)/Fe(II)(30 μM) treatment, less than 20% of gabapentin and metformin were removed after 1 h of treatment. The low removal rate for metformin could probably be explained by the fact that its molecular structure does not have the chromophores needed to absorb mercury-lamp wavelength light energy or some iron complexes with organic ligands (certain by-products such as oxalic acid) were formed, probably during the reaction time. These complexes exhibit higher absorbance and quantum yields than simple, aquated Fe-species. Therefore, they act as a light-absorbing species in the system, or as an inner-filter effect. The metformin self can also form stable complexes with metals [35].

3.4. Degradation of selected compounds in ultrapure water, lake water, and wastewater effluent

The water matrix plays an important role in pollutant photodegradation [24,36]. Therefore, we degraded micropollutants in different water matrices under UV_{254}/H_2O_2 and photo-Fenton treatments.

The lower yield of mean micropollutant removal was observed in WWTP effluent and lake water (Fig. 4). This was probably due to light absorption at 253.7 nm (22.7 m^{-1}) and competition for hydroxyl radicals by existing scavengers in water (carbonate, chloride, or humic-like substances).

Moreover, the WWTP effluent contains high chloride concentration (Table S2). Inorganic ions exert some coordinating effect over ferric ions [24,37], with the formation of thermodynamically favored complexes, such as $FeCl_2^+$, $FeCl^{2+}$, and $Fe[(SO)_4]_2^-$, reducing the photo-Fenton reaction's ability to recycle ferrous iron. In fact, the complexation of iron by chloride and sulfate ions could be primarily responsible for the process' diminished efficiency [38]. However, apart from inorganic ions, iron ions may also complex with certain organic compounds, especially those acting as polydentate ligands [39]. Therefore, in the water systems with more dissolved organic matter, the amount of oxidant available to react with organic compounds is lower, requiring higher oxidant doses to reach the desired pollutant removal level.

Table 3
Apparent first-order rate constants (min^{-1}) for photodegradation of selected compounds in MilliQ water at different Fe(II) concentrations.

Compounds	UV/H_2O_2 (300 μM)/Fe(II) (30 μM)	UV/H_2O_2 (300 μM)/Fe(II) (60 μM)	UV/H_2O_2 (600 μM)/Fe(II) (30 μM)	UV/H_2O_2 (600 μM)/Fe(II) (60 μM)
Gabapentin	0.0063	0.0033	0.0127	0.0051
Metformin	0.0038	0.0055	0.0040	0.0045
Metoprolol tartrat	0.0928	0.0669	0.0815	0.0645
Atenolol	0.0773	0.0610	0.0811	0.0562
Clarithromycin	0.0406	0.0360	0.0125	0.0094
Primidone	0.0423	0.0384	0.0318	0.0252
Methylbenzotriazole	0.1246	0.0894	0.1623	0.1080
Benzotriazole	0.3424	0.1859	0.3540	0.2408

3.5. Degradation products

No elucidation of all transformation products has been performed in this work. According to Collin et al. [40], the hydroperoxide of metformin, a covalent dimer of metformin, methylbiguanide, and the cyclic 2-amino-4-imino-5-methyl-1,3,5-triazine, were identified as the primary oxidation end-products. In all cases, the dimethylamino group was the reactive part of the metformin molecule.

In our study on degradation of eight micropollutants, guanylurea, phenol, oxalic acid, tartronic acid, glycolic acid, oxamic acid, and maleic acid, identified as fragmental oxidation products, were detected by scan-mode screening. Additionally, nitrogen-containing molecules were mineralized into NH_4^+ and mostly NO_3^-. Up to 300 μg/L of nitrate and ammonia were identified as final degradation products. Nitrate ions were more largely accumulated as the oxidizing power of the system increased, mainly due to the faster mineralization of the nitrogen-containing oxidation products.

As proposed for other phenols, we assumed that the degradation mechanism in the presence of H_2O_2 involved •OH radical attack. Furthermore, an oxidative ring-cleavage by hydroxyl-radical attack leads to aliphatic compound formation, such as HCOOH, CH_3COOH, and CH_3CHO, and finally to CO_2 [41,42].

3.6. Ecotoxicity testing of micropollutant mixtures after different treatments

To discover whether the treatments used can cause any toxic degradation products, the tests employing unicellular green alga, *C. reinhardtii*, were conducted. Toxicity experiments were performed on the mixture of the eight micropollutants of the mixtures (Fig. 5a) and of four micropollutants (Fig. 5b) after 10, 20, and 30 min of UV/H_2O_2 or UV/H_2O_2/Fe(II) treatments.

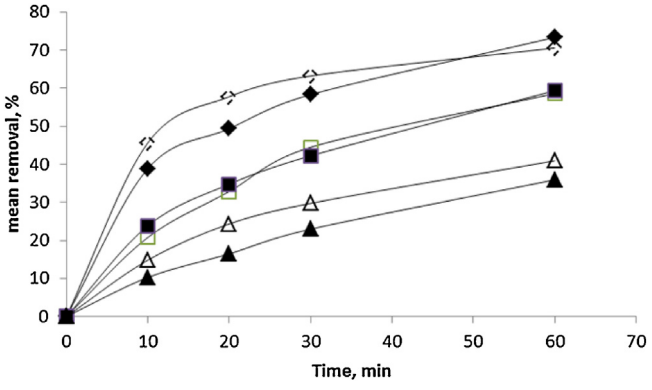

Fig. 4. Degradation of selected compounds in ultrapure water (♦), lake water (■), and wastewater effluent (▲) under UV_{254}/H_2O_2 (filled symbols) and photo-Fenton treatments (empty symbols). Initial conditions: 2 μM of each pollutant, 300 μM of H_2O_2, and 30 μM of Fe(II).

These mixtures were highly toxic for *C. reinhardtii* and no growth was observed in the undiluted samples, independent of the irradiation time. An EC50 of 21% [$IC_{95\%}$ = 16–27%] was found for the non-irradiated mixture, whereas an EC50 of 51% [$IC_{95\%}$ = 49–52%] was obtained for the mixture irradiated for 30 min. Further experiments were performed with the eight-micropollutant mixture in wastewater after 10, 20, and 30 min of UV/H_2O_2/Fe(II) treatment (Fig. 5a). Comparable results were found between the treatments

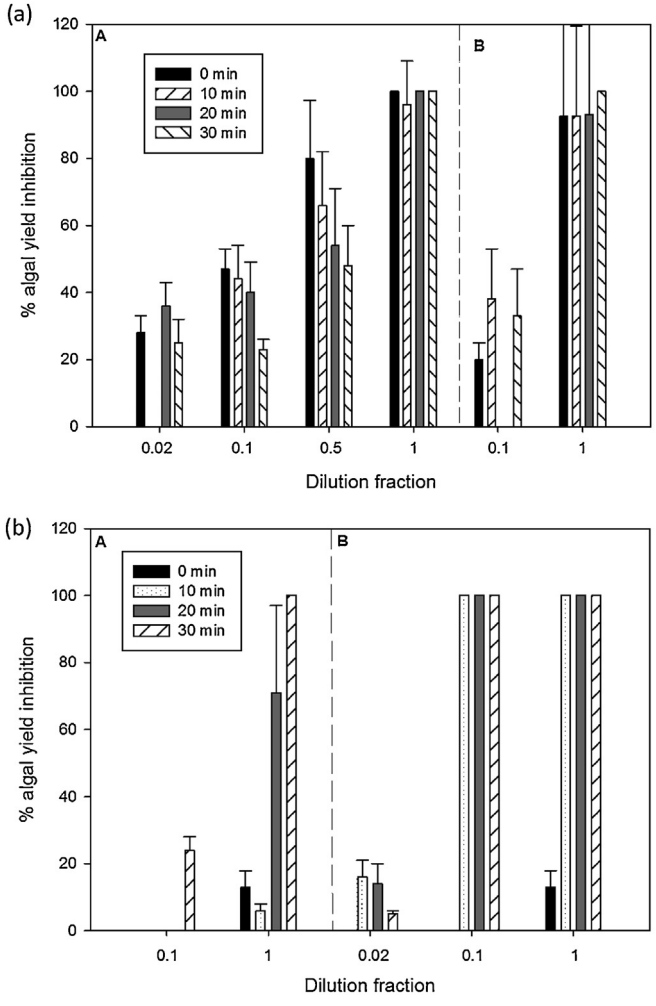

Fig. 5. Yield inhibition of *Chlamydomonas reinhardtii* exposed to a mixture of eight micropollutants (a) (atenolol, benzotriazole, clarithromycin, gabapentin, methylbenzotriazole, metformin, metoprolol and primidone; 2 μM of each compound) and a mixture of four micropollutants (b) (gabapentin, metformin, benzotriazole and diclofenac; 2 μM of each compound) (A) in milliQ water, and (B) wastewater under different treatments: A) UV/H_2O_2 (300 μM) – no Fe(II) added and (B) UV/H_2O_2 (300 μM) – 30 μM Fe(II) added. The *x*-axis represents the dilution fraction of the tested samples (undiluted by 1, dilution by 2–0.5, dilution by 10–0.1 and dilution by 50–0.02).

in milliQ water and wastewater. The eight-micropollutant mixture, diluted 10 times after 30 min of irradiation, inhibited algal growth by 25 ± 7% in experiments without Fe(II) and 33 ± 14% with addition of Fe(II).

The non-irradiated, four-micropollutant mixture (the two most stable pharmaceuticals, metformin and gabapentin), and two compounds (diclofenac and benzotriazole) selected for new legislation) inhibited algal yield by 13 ± 5%. However, an increase of growth yield to 71 ± 26% was observed after 20 min of UV/H_2O_2 (300 μM) treatment of the mixture (EC50 = 26%; $IC_{95\%}$ = 18–43%). The toxicity enhancement was exacerbated when irradiation was performed with the addition of 30 μM Fe(II). The 10-fold diluted mixtures after UV/H_2O_2 treatment decreased algal yield by 27 ± 8%, whereas UV/H_2O_2/Fe(II) treatment completely inhibited algal yield. The four-micropollutant mixture experiments showed 100% degradation of benzotriazole and diclofenac and only 10% of gabapentin and metformin within 30 min of UV/H_2O_2 treatment. Adding Fe(II) enhanced these compounds' degradation by 16% and 21%, respectively. The above results point out that the toxicity of a mixture upon different treatments highly depends on the micropollutants present. The results also suggest that the different substances' degradation products are more toxic for algae than the parent compounds themselves. Due to high gabapentin and metformin concentrations in surface waters and their stability during the treatments, these compounds must undergo further investigation and risk assessment. This is particularly true in the case of metformin, which aerobically biodegrades to guanylurea, which is stable against photo- and biodegradation [43]. In a recent study, the diclofenac degradation product, 2-[2-(chlorophenyl)amino]benzaldehyde, was found to be more toxic to the unicellular green alga *Scenedesmus vacuolatus*, than diclofenac itself, due to its higher hydrophobicity and thus likely higher bioaccumulation [44]. According to the European chemicals regulation REACH: "Consideration should be given to stable and/or toxic degradation products. Where such degradation products can occur, the assessment should give due consideration to the properties (including toxic effects and bioaccumulation potential) of the products that might arise" [1,45].

4. Conclusions

This study examined the degradation of eight micropollutants at the same molar concentration by UV_{254}, UV_{254}/H_2O_2, UV_{254}/H_2O_2/Fe(II), solar-simulated light//H_2O_2/Fe(II) in different water matrices. The initial H_2O_2 concentration, the effect of dissolved NOM, and common water constituents, influenced micropollutants' photodegradation. The results indicate that the oxidation rate increased in the presence of low-concentration H_2O_2 and Fe(II), except for metformin and gabapentin. Fenton and photo-Fenton employing sunlight simulation reached low micropollutant removal rates. The water matrix plays an important role on photodegradation reaction yields. The poor understanding of the NOM interactions and the unknown mechanism require more research.

The results highlight the need to consider proper wastewater treatment with regard to toxicity and persistent degradation product formation. The long lifetimes of water micropollutants suggest that wastewater streams require advanced oxidation technologies before being discarded into surface water. As our results reveal, whereas diclofenac and benzotriazole (as selected in new legislation) were completely removed in the first 10 min of reaction, less than 20% of gabapentin and metformin was removed after 1 h of treatment. Even if the data from WWTPs reports that metformin is relatively biodegradable, due to the high prescription volume worldwide of these pharmaceutical and high-influent concentrations, surface waters show increasing metformin concentrations in all sampling points, as reported in an earlier study [11]. Due to high gabapentin and metformin concentrations in surface waters and their stability during the treatments, these compounds must undergo further investigation and risk assessment. Moreover, these results are clearly relevant to ongoing debates regarding which organic micropollutants are to be subject to the required 80% elimination rates stipulated in new legislation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apcatb.2014.04.001.

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